PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Wesley Blackaby, et al.		
Serial No.:	10/593,950	Case: 21573YP	Art Unit: 1614
Filed:	May 10, 2007		
For:	HETEROARYL PIPERIDINE GLYCINE TRANSPORTER INHIBITORS		Examiner: Ricci, Craig D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF SCOTT WOLKENBERG

Sir:

I, Scott Wolkenberg, hereby declare the following:

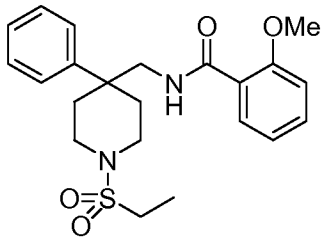
1. I have been employed by Merck & Co., Inc. beginning in 2003 and currently hold the title of Research Fellow in the Department of Medicinal Chemistry.

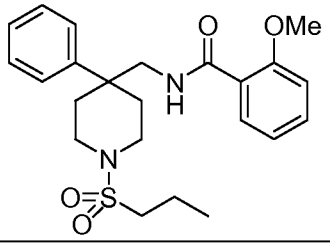
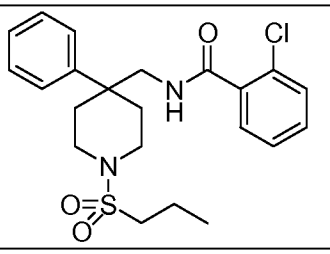
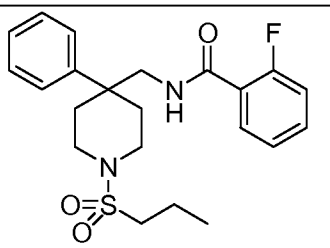
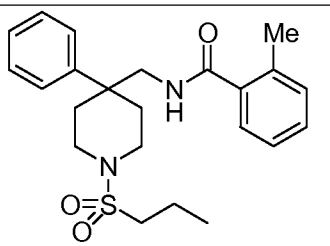
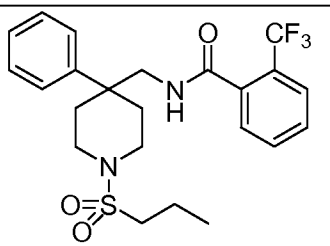
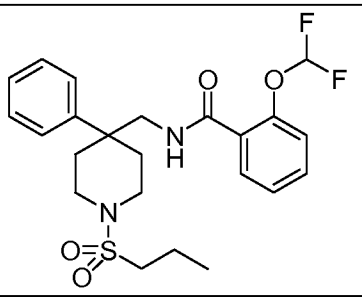
2. I am a joint inventor in the above-referenced patent application and have reviewed the Office action mailed October 23, 2009 issued therein.

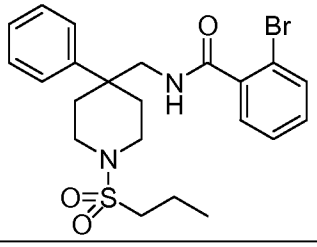
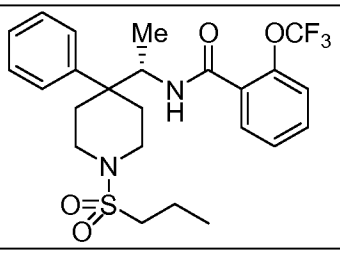
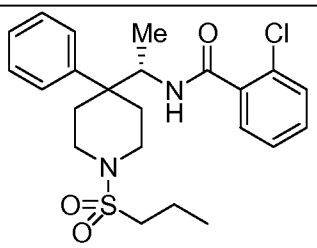
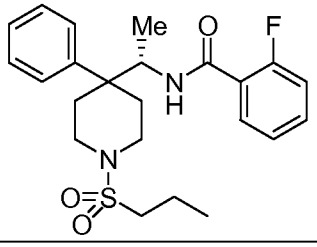
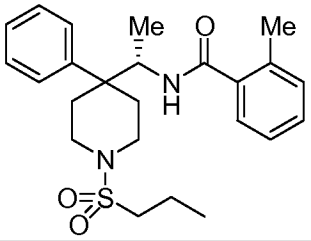
3. My job responsibilities at Merck included synthesizing compounds that inhibit glycine transporter GlyT1 and managing other chemists in synthesizing compounds for this target. As part of our efforts in this research, we routinely had the synthesized compounds tested in various *in vitro* and *in vivo* assays predictive of drug efficacy and safety.

4. As part of the research efforts described above, the compounds described in Table 1 below were tested for GlyT1 activity in accordance with the assay procedures described at page 18 of the above-captioned patent application. The compounds were also tested for potassium channels Kv1.5 and/or Kv1.3 inhibition in accordance with the assay procedures described in Bao, J.; Kotliar, A.; Kayser, F.; Parsons, W. H.; Rupprecht, K. M.; Claiborne, C. F.; Claremon, D. A.; Liverton, N.; Thompson, W. J. Heterocyclic Potassium Channel Inhibitors; U.S. Patent 6,303,637, Oct. 16, 2001; and/or according to this procedure: CHO cells that express Kv 1.3 or Kv1.5 are cultured in F-12 medium supplemented with 10% FBS, 100 g/mL penicillin, 100 U/mL streptomycin, 1000 g/mL geneticin (G418). The cells are harvested with trypsin and washed with F-12. 200 µL aliquot containing 100,000 cells/ml in F-12 (supplemented as above) is added per well to 96 well cell culture plate (assay plate) and the cells are allowed to grow for 48 hours at 37°C. The medium is removed and 200 µL of Rb Load Buffer (Aurora) is added for 3 hours at 37°C under 5% CO_2. The cells are washed 5 times with 200 µL Hank's Balanced Salt Solution (HBSS) (Gibco Cat#14025-092) followed by the addition of 100 µL HBSS containing tested compound or 0.5 % DMSO. After 10 min. 100 µL of HBSS containing 140 mM KCl is added and plate is incubated at room temperature for 5 min. Next, 150 µL of supernatant is transferred to a fresh 96 well plate and the remaining supernatant aspirated. 120 µL of Cell Lysis Buffer (Aurora) is added to the assay plate. Rb content is measured in samples of supernatant (SUP) and lysate (LYS) using ICR-8000 (Aurora). % FLUX=100%*(SUP/(LYS+SUP)). % INH=100%*(1-(A-B)/(C-B)), where A is % FLUX in the presence of tested compound, B is % FLUX in the presence of 10 µM L-836,885, C is % FLUX in the presence of 0.25% DMSO. The results are shown in Table 1 below. This table represents a selection of compounds screened versus both GlyT1 and Kv1.5 or Kv1.3.

Table 1

Structure	GlyT1 IC_{50} (nM)	Kv1.5 IC_{50} (nM)	Kv1.3 IC_{50} (nM)
	4211	Nd	340

Structure	GlyT1 IC_{50} (nM)	Kv1.5 IC_{50} (nM)	Kv1.3 IC_{50} (nM)
	135	297	320
	11	>25000	nd
	33	>25000	nd
	32	>25000	nd
	36	>25000	nd
	23	7200	nd

Structure	GlyT1 IC_{50} (nM)	Kv1.5 IC_{50} (nM)	Kv1.3 IC_{50} (nM)
	9	>25000	nd
	16	>25000	nd
	19	>25000	nd
	16	>25000	Nd
	26	>25000	Nd

5. The first two entries in the above table have methoxy groups at the 2-position of the benzamide. The first entry, which is Example 18 described in WO 2000/25786 ("Bao"), has an IC_{50} for Kv 1.3 of 340 nM. The second entry has IC_{50}s for Kv1.5 and Kv1.3 of 297 and 320 nM, respectively. The subsequent entries replace the methoxy group on the

benzamide with a different substituent at the same position. All such compounds lose potassium channel activity with IC_{50}s for Kv1.5 above 25000, the exception being CF_2-O-, which has an IC_{50} for Kv1.5 of 7200.

6. A key point raised in the Office action of October 23, 2009 is the bioisosterism of $-OCH_3$ and –Cl in the context of the compounds claimed in the instant patent application. *See* page 7 of the Office action stating: "Since the compounds taught by Bao et al. are "potassium channel inhibitors" (Title), the skilled artisan would have predicted that the well known bioisosteric modifications taught by Williams et al. and Pantani et al. – when applied to the potassium channel inhibitors taught by Bao et al. – would provide compounds having similar activity as potassium channel inhibitors, but with distinct pharmacokinetic profiles." As the data in Table 1 indicates, $-OCH_3$ and –Cl are not bioisosteric with respect to potassium channel activity. A selection of alternative potential bioisosteric compounds is also presented. Based on the above data, it is my opinion that the $-OCH_3$ group at the two position of the benzamide is unique in conferring potent potassium channel inhibition with respect to the claimed structural class of compounds.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statement made of information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

By /Scott E. Wolkenberg/
Scott E. Wolkenberg

Date: March 2, 2010